Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

Effect of three-year consumption of erythritol, xylitol and sorbitol candies on various plaque and salivary caries-related variables

Riina Runnel [a,*], Kauko K. Mäkinen [b], Sisko Honkala [c], Jana Olak [a], Pirkko-Liisa Mäkinen [b], Rita Nõmmela [a], Tero Vahlberg [d], Eino Honkala [b,c], Mare Saag [a]

[a] Department of Stomatology, Faculty of Medicine, University of Tartu, Estonia
[b] Institute of Dentistry, University of Turku, Finland
[c] Faculty of Dentistry, Kuwait University, Kuwait
[d] Department of Biostatistics, Faculty of Medicine, University of Turku, Finland

ARTICLE INFO

Article history:
Received 14 February 2013
Received in revised form
24 September 2013
Accepted 24 September 2013

Keywords:
Sugar alcohol
Dental plaque
Biofilm
Saliva
Clinical trial
Children

ABSTRACT

Objective: The objective of the present paper is to report results from oral biologic studies carried out in connection with a caries study.
Methods: Samples of whole-mouth saliva and dental plaque were collected from initially 7- to 8-year-old subjects who participated in a 3-year school-based programme investigating the effect of the consumption of polyol-containing candies on caries rates. The subjects were randomized in three cohorts, consumed erythritol, xylitol, or sorbitol candies. The daily polyol consumption from the candies was approximately 7.5 g.
Results: A significant reduction in dental plaque weight from baseline ($p < 0.05$) occurred in the erythritol group during almost all intervention years while no changes were found in xylitol and sorbitol groups. Usage of polyol candies had no significant or consistent effect on the levels of plaque protein, glucose, glycerol, or calcium, determined yearly in connection with caries examinations. After three years, the plaque of erythritol-receiving subjects contained significantly ($p < 0.05$) lower levels of acetic acid and propionic acid than that of subjects receiving xylitol or sorbitol. Lactic acid levels partly followed the same pattern. The consumption of erythritol was generally associated with significantly ($p < 0.05$) lower counts of salivary and plaque mutans streptococci compared with the other groups. There was no change in salivary *Lactobacillus* levels.
Conclusion: Three-year consumption of erythritol-containing candies by initially 7- to 8-year old children was associated with reduced plaque growth, lower levels of plaque acetic acid and propionic acid, and reduced oral counts of mutans streptococci compared with the consumption of xylitol or sorbitol candies.

* *Corresponding author at*: University of Tartu, Department of Stomatology, Raekoja plats 6, Tartu 51003, Estonia. Tel.: +372 731 9856; fax: +372 731 9856.
E-mail address: riina.runnel@ut.ee (R. Runnel).
0300-5712/$ – see front matter
http://dx.doi.org/10.1016/j.jdent.2013.09.007

1. Introduction

Ancillary studies carried out as part of clinical trials aimed at investigating caries prevention have provided important information concerning oral physiologic processes and helped outline the mechanism of prevention. Examples of such clinical trial/ancillary study efforts include the Turku and the Belize caries prevention programmes which studied the caries-preventive effects of xylitol and sorbitol.[1,2] A recent clinical study implemented in Tartu, Estonia, offered a further opportunity to investigate the possible caries-preventive effect of a homologous series of dietary sugar alcohols, *viz*. erythritol, xylitol, and sorbitol.[3] In this study, the effect of three-year consumption of erythritol, xylitol, and sorbitol candies was investigated in a child population initially consisting of 485 primary school children from the region around Tartu, southeastern Estonia. The main finding of this study was that the number of dentine caries teeth and surfaces in the mixed dentition were lower in the erythitol group than in the xylitol or sorbitol groups.

The objective of the present paper is to report results from oral biologic studies carried out in connection with the above described caries prevention study in children. The ancillary tests focused on salivary and dental plaque levels of mutans streptococci, on salivary *Lactobacillus* levels, and on chemical analyses of dental plaque. Information was also obtained from plaque gravimetry and salivary flow rates during the study years.

2. Materials and methods

2.1. Study design and general procedures

The Tartu study was set up as a double-blind, randomized, placebo-controlled prospective intervention trial. The study design, study population, and treatment of the subjects have been described in detail elsewhere.[3–5] The overall flow chart of the trial is shown in Fig. 1 with information on the number of subjects. In summary, first- and second-graders (7 and 8 years old; $n = 485$) of the participating Tartu area public school classes were randomly divided into three groups of 156–165 children who consumed either erythritol-, xylitol-, or sorbitol-containing candies on school days over a period of three years. The list of all classes from all participating schools was used as a sample frame. Inside the schools, the 1st grade pupils were allocated into a different group than the 2nd graders to reduce school bias. There were about 200 school days per calendar year. Each child consumed four 0.7 g candies three times per school day, the daily intake of each sugar alcohol amounting to approximately 7.5 g. No side effects were expected with that amount. One piece of candy contained about 90% of erythritol, xylitol, or sorbitol. Otherwise the contents of the candies were similar. Candies were manufactured and provided by Cargill R&D Centre Europe. The teachers distributed and supervised the use of the products before the first lesson in the morning (8 am), immediately after the school lunch (10.30 am), and at the end of the school day (1.30 or 2.15 pm). They were trained by RN before start of the intervention. A group representing the investigating team made three annual site visits to the schools during the intervention to enhance compliance of subjects to the study. Most of the children also confirmed their compliance, when questioned by the researchers during the annual examinations.

The subjects were examined four times during the trial: at baseline in 2008 and in the following years once a year (2009–2011). All examinations, including the plaque and saliva collections in question here, were carried out between January and February. The examinations were conducted at standard dental units of the Department of Stomatology, University of Tartu. The International Caries Detection and Assessment System (ICDAS II) was used in the clinical examinations.[6] At the baseline, subjects were blindly assigned to examiners. The subject-examiner assignment was fixed for the duration of the study. Double-blind clinical examinations of the children in all groups were completed by four trained and calibrated investigators (EH, SH, JO, RR). The number of children studied in all four clinical examinations reduced from 165 to 122 in erythritol group, from 156 to 126 in xylitol and from 164 to 126 in sorbitol groups. All pupils received dental health education on oral hygiene and diet in connection with the annual examinations. Each half year, every child was also given a toothbrush and fluoride toothpaste (Colgate Total® with 0.24% sodium fluoride; and sorbitol as a sweetener). At each examination, children were recommend to brush their teeth more than once a day.

An endpoint control group (an additional comparison group) from the same sample frame was drawn after the above examinations of the pupils in the three intervention groups. This group was examined in May 2011 in a way identical to all the previous examinations, following the completion of the fourth and final examinations of the original three intervention groups. The same number of children ($n = 162$) within the same age groups were thus examined to enable comparisons between the intervention groups and the endpoint comparison group.

The entire study was conducted according to the ethical principles of the Declaration of Helsinki. The study protocol (166/T-7) was approved by the University of Tartu Research Ethics Committee. Approvals of the School Management Authority and school principals were also received. The study was listed to the register of clinical trials (www.clinicaltrials.gov) at initiation as Clinical Trials.gov Identifier NCT01062633. Informed consent was obtained for all study subjects from the parents/caretakers.

2.2. Sample collection and microbiologic methods

Salivary and plaque counts of *Streptococcus mutans* (below collectively called mutans streptococci, SM) and salivary counts of *Lactobacillus* (LB) were determined in connection with all clinical examinations by means of the Orion Diagnostica (Espoo, Finland) Dentocult® SM and the Dentocult® LB Dip Slide procedures, respectively. Prior to each visit for plaque and saliva collection, the subjects were instructed to maintain regular, accustomed dietary habits. They were allowed to eat breakfast in the morning and those children who had examination in the afternoon had a light lunch. They

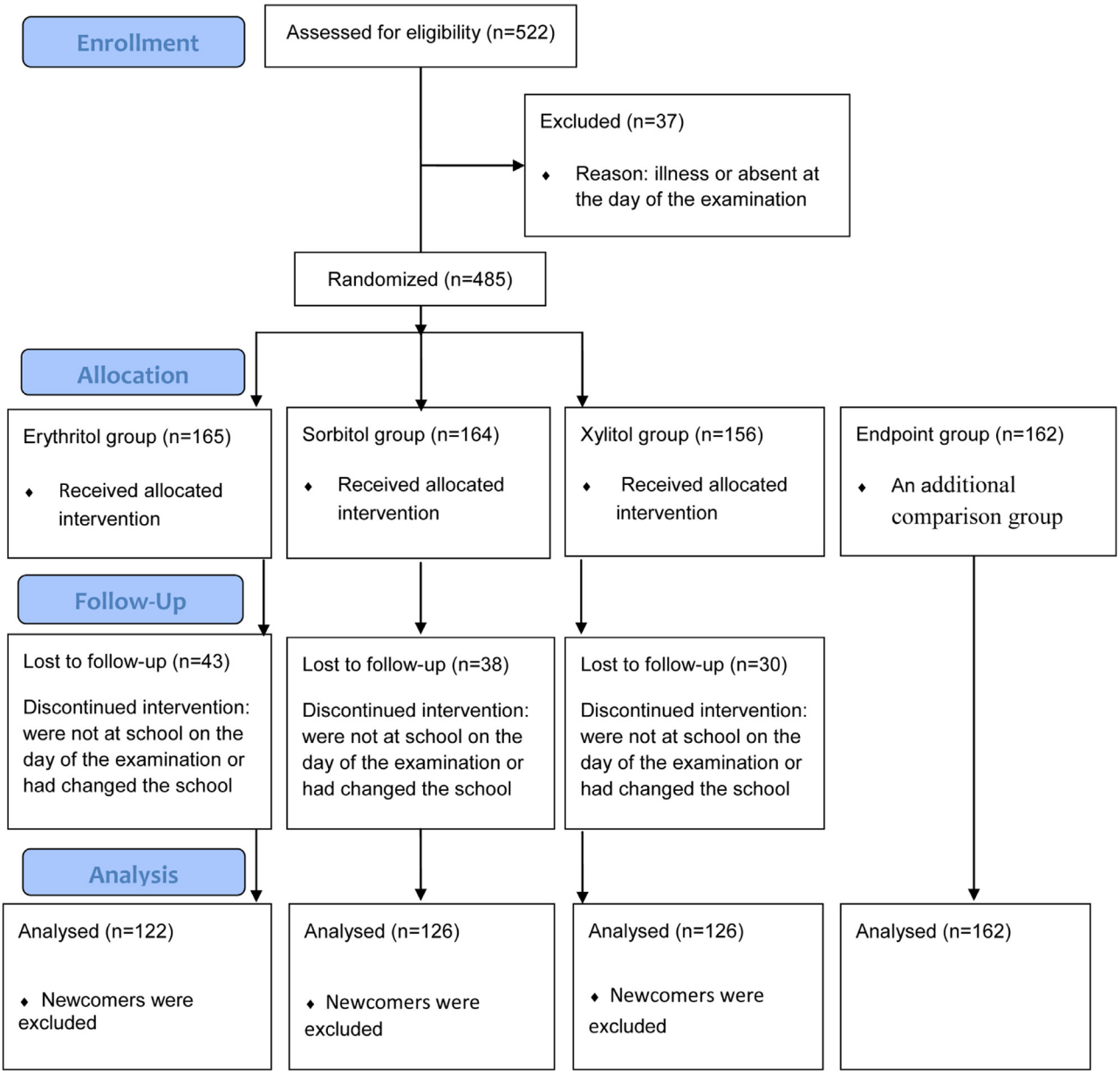

Fig. 1 – The flow chart of the study.

were advised and reminded by teachers not to brush their teeth in the morning of the examination.

The sequence of the procedures was as follows: four plaque samples for the 'Site Strip Test' for SM were obtained from each subject using disposable Quick-Stick® microbrushes (Dentonova AB, Huddinge, Sweden). These samples (designated below as SM count of each quadrant [SM 1–4]) were obtained during each examination by gently stroking the brush along the proximal surfaces near the gingival margin between the 1st permanent and the 2nd primary molar in each quadrant. If the 2nd primary molar was missing, the sample was taken from the mesial surface of the 1st permanent molar. The average percentage of total plaque removed from the tooth surfaces by the microbrush was estimated to be at 3%. Each brush was then successively rubbed against the corresponding roughened, numbered site of the cultivation strip of the Dentocult® 'Site Strip' included in the test package. Accordingly, four separate plaque samples, each representing one of the four quadrants, were obtained from each subject. Subsequently, the cultivation strip was placed in the culture medium supplied by the manufacturer. The same cultivation tube was used for the study of the salivary SM levels, as per the manufacturer's instructions. Saliva for this assay was thus not separately collected, since the procedure presumed rotating the sample collection spatula in the mouth of the subject for 10 s. The quantification of SM was based on the use of four scores (0, 1, 2, and 3), as described in the manufacturer's manual. This procedure included a timed 2-minute chewing of a piece of paraffin prior to the test proper. The saliva collected during the 2-min chewing was used for the determination of the LB count and the volume of saliva (for the assessment of the salivary flow rate). In the LB procedure, the number of

aerobically cultured aciduric bacteria per ml of saliva was assessed as per instructions in the manufacturer's manual (scored 0, 3, 4, 5, or 6).

Following the above procedures, the investigators collected all available dental plaque from all available tooth surfaces during a timed 3-min period. Plaque was collected using a probe and a disposable weighing pan. The fresh weight of each total plaque sample was immediately determined using an analytical balance. The plaque samples were subsequently and quantitatively suspended in sterile 0.9% NaCl in an iced water bath. Because the ensuing HPLC-based chemical analyses could not be conducted on individual plaque samples, plaque pools were made at each examination by combining plaque from the same subjects. Accordingly, a total of 15–16 plaque pools were normally obtained at each examination, each pool representing ten subjects at baseline (at later visits, a few pools were slightly smaller owing to absent subjects). The suspensions of pooled total plaque were frozen in dry ice and stored at −80 °C for chemical analyses which were carried out at Cargill laboratories (Vilvoorde, Belgium).

2.3. Chemical procedures

The frozen dental plaque samples were thawed and immediately homogenized for 1 min with a Vortex mixer at maximum speed. The homogenates were centrifuged for 5 min at 15,000 × *g* (+4 °C). Each supernatant was passed through a disposable 0.45-μm filter, while the pellets were returned to the freezer (−80 °C). A 200-μl aliquot of each filtrate was inserted into an HPLC glass vial that was immediately placed in the auto-sampler at +5 °C. An aliquot of the remaining filtrate was diluted 50-fold with HPLC-grade water for protein analysis. The rest of the filtrate was returned to the freezer. Consequently, the objects of the present chemical and HPLC analyses were aqueous plaque extracts containing plaque ingredients soluble in cold 0.9% NaCl.

For the determination of plaque sugars, organic acids, and polyols, the named compounds were separated by means of HPLC with the following specifications: pre-column: Bio-Rad Cation-H cartridge (kept at room temperature); column: 2 Shodex KC-811 H^+ form) in series; temperature: 75 °C; eluent: 0.01 M H_2SO_4 (kept at 60 °C); flow rate: 0.5 ml/min; injection: 50 μl; detection: differential refractive index, set at 16×; data acquisition: Atlas-8 from Thermo Scientific. The calibration of the system and the quantification of the compounds were carried out by means of the external equilibrium mode.

For the analysis of plaque calcium, the following specifications were used: pre-column: Dionex IonPac CG12A (kept at room temperature); column: Dionex IonPac CS12A; temperature: room temperature; eluent: 20 mM methanesulfonic acid (at room temperature); flow rate: 1.0 ml/min; injection: 25 μl; suppressor: Cation Self-Regenerating Suppressor, CSRS II; detection: Conductivity, set at 300 μS; data acquisition: Atlas-8 from Thermo Scientific. The calibration of the system and the quantification of calcium were carried out by means of the external calibration mode. Plaque soluble proteins were determined using the Bio-Rad procedure and the Mitsubishi TN-05 instrument. Plaque protein levels were expressed in g per 100 g of fresh plaque.

The sources of the chemicals used were as follows: acetic acid (glacial, 100%, anhydrous), ethanol (absolute), calcium chloride dihydrate (min. 99.5%), methanesulfonic acid, sulphuric acid, and saline (physiological NaCl solution) were obtained from Merck. L-(±)-lactic acid (98%), propionic acid (99%), maltose monohydrate (min. 98%), maltotriose (min. 95%), and xylitol (min. 99%) were products of Sigma. D-(±)-glucose (anhydrous) and sorbitol (≥99.5%) were obtained from Fluka. Glycerol (99%) was purchased from Riedl-de-Haën and erythritol (≥99.9%) was manufactured by Cargill. The reference for protein (nitrogen) determinations was the Nitrogen Standard Solution (Ion HIQU) of Chem-Lab N.V., and contained 1000 μg/ml of nitrogen in the form of NH_4Cl.

2.4. Data management and statistical procedures

The changes SM and LB counts, plaque weights and chemical plaque data compared to baseline within groups during the course of the study were analyzed using the Wilcoxon Signed Rank test. Bonferroni-corrected *p*-values were used. Differences of above parameters between groups were analyzed using the Kruskal–Wallis test and pair-wise comparisons were made using the Mann–Whitney *U*-test with Bonferroni correction. Statistical analyses were performed using SAS System for Windows, release 9.2 (SAS Institute Inc., Cary, NC, USA) and *p*-values less than 0.05 were considered statistically significant. According to the manufacturer's manual, the Orion Diagnostica SM procedure uses classes 0, 1, 2, and 3. These values correspond to ≤10,000 CFU/ml, 10,000–100,000 CFU/ml, 100,000–1,000,000 CFU/ml, and ≥1,000,000 CFU/ml, respectively. In the present study, the *p*-values for SM data were computed and the statistical inference performed using the above nonparametric methods. Although ordinal data have been usually presented using frequencies (and percentages) or median (and interquartile range), the SM values have been quite often described as mean ± SEM, which is the procedure employed in the present study. Using the mean ± SEM values, one can more readily infer the direction of the difference between groups.

3. Results

3.1. Salivary SM and LB counts and plaque SM counts

The bacterial counts between groups did not differ at baseline (2008). There were statistically significant differences between the groups in saliva and plaque SM counts after 3 years (Table 1). The salivary SM counts and plaque SM counts in quadrants 1 and 2 were significantly higher in the sorbitol group than in the erythritol group ($p < 0.05$). In the erythritol and xylitol groups, the differences within the groups were statistically significant during the follow-up years, in 2008–2011, for all parameters apart from LB in the xylitol group (not shown) ($p < 0.01$).

3.2. Plaque fresh weights

The total plaque weights determined annually are shown in Table 2 for the different groups. There was no significant

Table 1 – The mean (and SEM) counts of salivary SM, plaque SM by quadrants 1–4, and salivary LB levels at the baseline and during a three-year intervention between the intervention groups.

	Erythritol				Sorbitol			Xylitol			
	Baseline (n = 165)	1-year (n = 142)	2-years (n = 132)	3-years (n = 122)	Baseline (n = 164)	1-year (n = 149)	2-years (n = 137)	3-years (n = 126)	Baseline (n = 156)	1-year (n = 145)	2-years (n = 132)	3-years (n = 126)
Saliva SM	1.76 (0.08)	1.56 (0.08)	1.58 (0.08)	1.21 (0.09)	1.74 (0.08)	1.44 (0.08)	1.62 (0.09)	1.65 (0.08)	1.85 (0.08)	1.61 (0.08)	1.68 (0.08)	1.47 (0.09)
Plaque SM1	1.55 (0.08)	1.34 (0.08)	1.38 (0.09)	1.11 (0.09)	1.48 (0.08)	1.44 (0.08)	1.35 (0.08)	1.45 (0.09)	1.68 (0.08)	1.41 (0.08)	1.41 (0.08)	1.24 (0.09)
Plaque SM2	1.52 (0.09)	1.41 (0.08)	1.29 (0.08)	1.10 (0.09)	1.60 (0.08)	1.45 (0.08)	1.44 (0.08)	1.40 (0.09)	1.77 (0.08)	1.50 (0.08)	1.45 (0.08)	1.37 (0.09)
Plaque SM3	1.66 (0.09)	1.57 (0.09)	1.43 (0.09)	1.23 (0.10)	1.67 (0.09)	1.54 (0.09)	1.42 (0.10)	1.33 (0.09)	1.80 (0.09)	1.48 (0.09)	1.47 (0.09)	1.41 (0.10)
Plaque SM4	1.69 (0.09)	1.36 (0.09)	1.49 (0.09)	1.13 (0.09)	1.60 (0.08)	1.36 (0.09)	1.48 (0.09)	1.31 (0.10)	1.78 (0.08)	1.34 (0.09)	1.39 (0.09)	1.23 (0.09)
Saliva LB	4.36 (0.13)	3.94 (0.16)	3.37 (0.17)	3.46 (0.18)	4.47 (0.13)	4.11 (0.15)	3.81 (0.16)	3.52 (0.18)	4.36 (0.13)	4.28 (0.14)	3.82 (0.17)	3.93 (0.17)

Significant p-values: the 3rd year saliva SM $p = 0.0019$, plaque SM1 $p = 0.0256$, plaque SM2 $p = 0.0280$

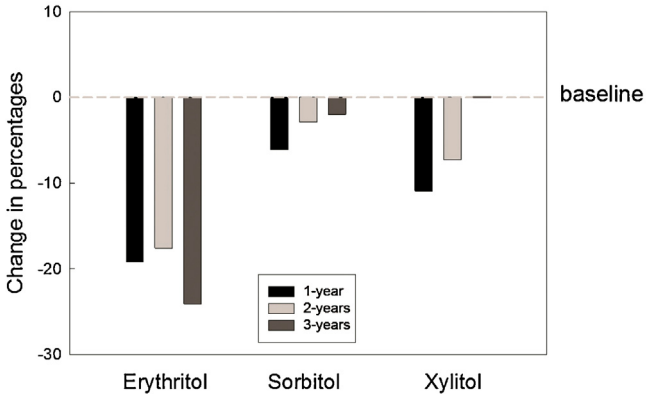

Fig. 2 – The reduction of mean plaque fresh weights during a three-year intervention, compared with baseline values.

difference between the groups at baseline in 2008. After 3 years, the lowest total plaque values were measured in the erythritol group. During most of the follow up years, plaque levels were significantly lower in the erythritol group ($p < 0.05$ at each year from baseline, with tendency after 2 years). In the sorbitol and xylitol group no changes were observed from baseline at any of the follow-up years. The above changes are more graphically illustrated in Fig. 2, where the percentage changes (positive or negative) in the mean plaque weights measured after the 1st, 2nd, and 3rd year are compared with the baseline. The largest reductions in total plaque weights over time were observed in the erythritol group.

3.3. *Salivary flow rate*

As part of the monitoring of the subjects' basic oral physiologic status, their salivary flow rates were assessed during the intervention. As expected, the values increased significantly ($p < 0.001$) in each group as the subjects grew older. The mean stimulated flow rates of the entire child cohort were approximately 1.1 ml/min (baseline), 1.5 ml/min (one-year), 1.8 ml/min (2-year), and 2.0 ml/min (3-year). No differences were found among the groups at any of the above time points (data not shown).

3.4. *Chemical analysis of aqueous plaque extracts*

The lowest concentrations of acetic acid and propionic acid, and partly of lactic acid, were found after the 3rd year in the erythritol group; the differences were significant ($p \leq 0.05$) for acetic acid and propionic acid among all study groups, while similar difference for lactic acid appeared only between erythritol and xylitol (Fig. 3). When comparing acid levels according to study years in the sorbitol group, the only statistically significant differences ($p \leq 0.05$) were found for lactic acid between the 3-year and the other time points. In the xylitol group, there were no significant differences between study years.

The levels of calcium present in the aqueous plaque extracts normally ranged between 200 μg and 600 μg per g of fresh plaque while those of glucose normally remained within one mg and 16 mg per g of plaque (not shown). The levels of

Table 2 – The mean (and SEM) of fresh weights (in mg) of total plaque at baseline and subsequent study follow up years.

Erythritol group (n = 165)				Sorbitol group (n = 164)				Xylitol group (n = 157)			
Baseline	1-year	2-years	3-years	Baseline	1-year	2-years	3-years	Baseline	1-year	2-years	3-years
10.42 (0.65)	8.47 (0.66)	8.64 (0.73)	7.96 (0.64)	9.99 (0.55)	9.38 (0.58)	9.69 (0.74)	9.29 (0.69)	9.61 (0.60)	8.61 (0.58)	8.96 (0.68)	9.72 (0.72)

No significant differences between groups. Indicative differences (the p values approached significance): year 1, erythritol vs. sorbitol, $p = 0.058$; year 2, erythritol vs. sorbitol, $p = 0.086$; year 3, erythritol vs. sorbitol, $p = 0.10$. No changes within groups over time for sorbitol and xylitol. Changes within group for erythritol were significant ($p < 0.05$) between 1st and 3rd years vs. the baseline (indicative difference for the 2nd year vs. the baseline.

glycerol normally ranged between 100 μg and 400 μg per g of plaque and the values did not differ between study years and study groups (not shown). There were no consistent or significant differences between treatments, or over time, in the analyses of calcium and glucose. Plaque protein levels normally ranged from 1.2 mg/100 g plaque to 1.6 g/100 g plaque, and remained virtually unchanged during the 3-year trial in all groups; there were no differences between the groups (not shown).

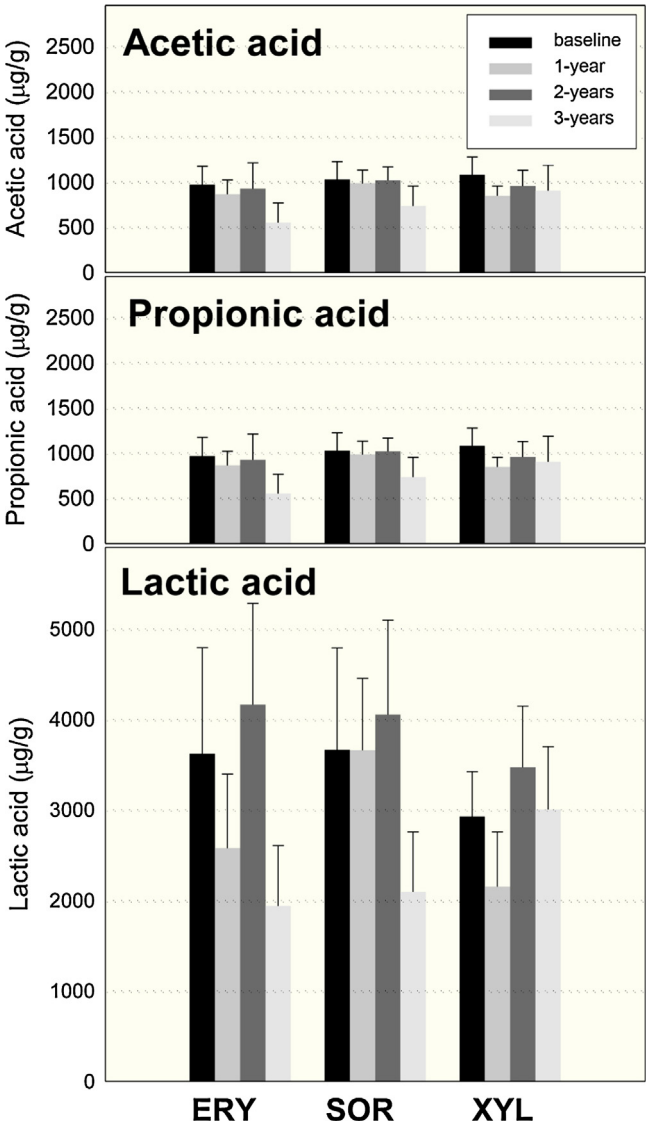

Fig. 3 – Concentration of acetic acid, propionic acid, and lactic acid in the dental plaque in the study groups at the baseline and during a three-year intervention. The values shown are mean ± SD.

The concentrations of sorbitol varied very significantly, i.e., from almost zero to 700 μg per g of fresh plaque (not shown). This result may imply considerable variation in the time interval between candy or meal consumption and plaque sampling. However, the groups did not differ meaningfully at any point. Analysis of erythritol and xylitol resulted in even wider variations; the groups and study years did not differ.

3.5. *Results of the endpoint comparison group*

The fourth study group formed three months following the termination of the intervention proper, was investigated in May 2011 using the same oral biologic parameters as for the other groups. The results were compared with the endpoint (2011) values obtained with the intervention groups, and are summarized below.

The mean salivary SM counts of the endpoint group were significantly higher than those determined for the erythritol and xylitol groups at endpoint ($p = 0.014$ and 0.034, respectively). There was no difference between the sorbitol group and the endpoint group. The mean plaque SM counts of all four quadrants were significantly ($p < 0.05$) higher in the endpoint group than in the intervention groups (not shown). The salivary LB counts were also higher in the endpoint group than in the other groups, although the differences were not significant. The comparison group's mean ± SEM weight of total plaque was 9.16 ± 0.76 mg and the salivary flow rate 1.46 ± 0.75 ml per min.

The chemical analyses of total plaque in the endpoint group generally produced results similar to those measured in the intervention groups. However, the concentration of acetic acid and propionic acid were significantly ($p < 0.05$) lower in the erythritol group than in the comparison group, whereas the mean lactic acid values were very similar (data not shown).

4. Discussion

4.1. *General observations about biological examinations of dental plaque*

Dental plaque exhibits several major properties related to dental caries: (1) plaque provides potentially harmful bacteria on tooth surfaces; (2) plaque adheres to tooth surfaces; a greater plaque mass may be potentially more harmful than a thin, newly formed plaque integument as it forms a barrier to

acid-neutralizing substances in saliva; (3) plaque is biochemically capable of rapidly metabolizing dietary carbohydrates to acids. The biological markers included the oral counts of SM and LB, plaque gravimetry, and concentration levels of dental plaque compounds believed to reflect the biochemical and cariologic status of plaque. Since there were no side effects, the usage of the saliva stimulants was similar in all study groups.

4.2. *Plaque mass, SM, and comments on differences between the tested alditols*

Most oral biologic studies involving the use of xylitol- or sorbitol-sweetened products have revealed distinctive differences between these polyols. Xylitol has almost consistently been shown to reduce the mass and adherence of dental plaque on tooth surfaces, and to reduce the growth of SM on tooth surfaces; this reduction has occasionally also been reflected in whole-mouth saliva SM levels. Plaque mass and adherence, and plaque SM level, can be regarded as surrogate end points of dental caries. The few oral biologic studies carried out with erythritol have suggested that this alditol may affect these endpoints similarly to xylitol, or its effect may even exceed that of the latter.[7–9] In the present three-year intervention, only erythritol reduced the amount of dental plaque consistently during the entire follow-up. There were no significant changes over time in the sorbitol (control) and xylitol groups over time during the intervention. What is more, detectable and more consistent reduction in plaque levels of acetic acid, propionic acid, and lactic acid occurred in the erythritol group, and the salivary and plaque SM counts determined for the 1st and 2nd quadrant (upper teeth) were significantly lower in subjects who received erythritol, compared with other groups.

The superiority of erythritol in the above measurements can be evaluated from the standpoint of its generally known molecular properties that differentiate it from pentitols and hexitols, i.e., the erythritol molecule's significantly smaller molar mass (122.1 g/mol) and its consequently higher "mobility" in biological systems.[9–11] The smaller molar mass of erythritol should make it more permeable and more active in biological environments than its larger homologues xylitol and sorbitol, with their molecular mass of 152.1 g/mol and 182.2 g/mol, respectively. Although erythritol does share most of the general polyol properties with xylitol and sorbitol, it is likely that the erythritol-associated plaque effects can be partly interpreted in terms of the erythritol molecule's smaller molar mass and its general osmoregulator role in biological environments. Previous papers have shown that erythritol is an effective hydroxyl radical scavenger[10,11] and that it retards the growth of certain SM strains more effectively than xylitol or sorbitol.[7] Although xylitol, too, has pronounced free-radical quenching ability, it is still possible that erythritol provides certain cariologic advantages over xylitol and sorbitol. The final mechanism of action may turn out to be more complex provided the results obtained with a chronic wound biofilm model can be used as a point of comparison: erythritol preferentially inhibited *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while xylitol preferentially targeted *P. aeruginosa*.[12] These results speak for pronounced selectivity in effects of alditols on human pathogens. Contrasting results of erythritol and xylitol were obtained with SM: compared with xylitol, erythritol at low concentrations had a weaker effect on the growth and acid production of SM, but had a stronger effect at high concentrations.[13]

In a comparative study by Elseviers et al.,[14] it transpired, inter alia, that D-erythrose and L-erythrulose (the aldose and ketose forms corresponding to erythritol) displayed anticariogenic properties in terms of their inhibitory action on SM ATCC 25175. These sugars turned out to be effective growth inhibitors even when blended with glucose, and certain blends produced no lactic acid upon fermentation. A later study showed that certain mixtures of erythritol with sorbitol or xylitol effectively retarded the growth of SM in interproximal dental plaque.[8] An aspect of previous research dealing with erythritol suggested that erythritol was neither utilized as a substrate for lactic acid production nor for plaque formation of SM and certain oral microorganisms.[15] Erythritol was also not utilized for water-insoluble glucan synthesis or cellular adherence by glucosyltransferase from SM PS-14 and *S. sobrinus* 6715. Combined, these results suggest that four-carbon carbohydrates and alditols may constitute a promising next-generation group of natural and physiologic sugar substitutes.

The failure of xylitol to reduce plaque and SM levels consistently during the present follow-up study is difficult to explain in view of previously reported, generally recognized effects. About 90% of all plaque studies with xylitol and sorbitol have shown xylitol to reduce plaque mass and SM counts, while the use of sorbitol has normally been associated with no change in these parameters, or even increased plaque quantity and bacterial levels. One possible reason may be the fact that treatment during the span of the study was relatively mild: (1) test products were only consumed 3 times a day with the last consumption just before children left school around 2 pm, (2) test products were only consumed during weekdays and not during the weekend, and (3) test products were not consumed during 2 months of school vacation.

The presence of polyols in plaque did not correlate with group assignment; salivation was considered to have caused a relatively effective clearance of occasional erythritol and xylitol residues from plaque. These polyols may not be regarded as constitutive plaque metabolites in the same way sorbitol is known to be involved in plaque carbohydrate metabolism.

The present study subjects displayed relatively high LB counts, which is in agreement with a previous study reporting high levels of salivary LB in Estonian schoolchildren.[16] Increased salivary LB levels have previously been ascribed to the presence of untreated dentine caries. Some studies have indeed demonstrated a correlation between high levels of LB in whole-mouth saliva and dentine caries[17], and long-term use of xylitol chewing gum in a school programme did reduce the salivary LB levels compared with controls (Belize and China studies).[17–19] The present trial showed a significant difference between the intervention groups in the number of dentine carious teeth and surfaces (in the primary dentition) at the last two visits (after 2nd and 3rd year).[3–5] This difference was not reflected in the LB measurements, however. This may have resulted from the relatively low overall number of untreated dentine caries lesions in the present child cohort.

4.3. *Plaque acids*

One of the pathogenic properties of dental plaque results from its biochemical capability to metabolize dietary carbohydrates to acids that can demineralize the tooth and contribute to the formation of an acidic microenvironment within plaque for increased growth of aciduric organisms (such as lactobacilli). A variety of organic acids have been detected in dental plaque. In the present study, acetic acid, propionic acid, and lactic acid were selected to represent acidic end products of plaque bacterial metabolism (i.e., surrogate markers of caries), in which these acids can be visualized as being formed after hexose-to-pyruvic acid conversion of bacterial metabolism. Although the acid levels varied remarkably over time in all experimental groups, the plaque collected from erythritol-using subjects showed most significant reduction in these plaque acid levels. It was noted already in the early history of erythritol research that acetic acid bacteria do not ferment erythritol and that yeast is totally incapable of metabolizing erythritol.[20] The same appears to apply to the lactic acid bacteria harboured in the oral cavity.

4.4. *Plaque mass-to-calcium relationship*

The present study showed that the plaque levels of protein and calcium remained within a relatively normal range during the intervention. The fact that the concentrations of plaque protein, which may be considered to represent more stable, basic plaque constituents, remained within a quite narrow range during the trial, suggests that the results of other chemical analyses reflected the metabolic state of the present plaque samples relatively well. The rationale behind the present calcium determinations was to investigate the possibility that the present alditol usage would result in similar Ca-to-plaque ratios as in four previous studies.[9] In these studies, plaque calcium levels increased in subjects who had consumed xylitol. These phenomena were assumed to be related to the complexation of calcium with alditols, but more specifically resulting from an increase in plaque protein levels in the dental plaque of xylitol-consuming subjects. Dental plaque may thus be regarded as a calcium reservoir which releases calcium to aid repair.[21] The present analyses did not reveal the same Ca:plaque (or Ca:protein) relationship in any of the test groups analyzed after each visit, as had been found in the four studies referred to above. There were no differences between the experimental groups. It is possible that the present polyol treatment was too mild for the above Ca:plaque ratios to occur.

4.5. *Comparison with the previous 6-month study*

A previous short-term study carried out in the same geographic area with teenage subjects revealed significant differences in several oral biologic parameters between the same alditols investigated in the present trial.[7] In the short-term study, the use of erythritol and xylitol was associated with a statistically significant reduction in the plaque and saliva levels of SM and in the growth of dental plaque. The use of sorbitol did not affect these oral biologic variables in the same way. The teenage subjects received 6.72 g of the above polyols in the form chewable tablets, and additionally used twice daily a dentifrice containing 34.5% (w/w) of the same polyols, increasing the overall daily polyol usage to about 7.0 g per subject, i.e. to about the same level as in the present three-year intervention trial. This polyol usage continued uninterrupted over the entire six-month period. The tablets were consumed in six separate episodes per day, the overall daily frequency of exposure of the dentition to the tested polyols thus being eight. This polyol usage covered most of the waking period of the subjects, whereas in the present trial the test items were normally consumed daily within a relatively short six-hour period. Therefore, the study designs of these two studies are disparate. In spite of the differences between study designs, the use of erythritol candies was associated with significantly decreasing plaque amounts during both the short-term and the long-term intervention. Also, the significant plaque weight reduction observed for erythritol compared to xylitol and control in this long-term intervention is consistent with the finding in the short-term intervention, where a significantly higher plaque weight reduction was observed for erythritol compared to xylitol and sorbitol.

5. Conclusions

The present study showed that plaque levels of acetic acid, propionic acid, and lactic acid were significantly lower after the 3rd year in the erythritol group compared with xylitol and sorbitol groups. The amount of dental plaque was consistently reduced only in the erythritol group during the entire follow-up. Also the saliva SM counts and the plaque SM counts in the 1st and 2nd quadrant (upper) were lower in the erythritol group than in the other intervention groups. These results are in congruence with the reduction in the number of dentine caries teeth and surfaces reported in the clinical paper of this series.[3–5]

Acknowledgement

The funding provided by Cargill R&D Centre Europe (Vilvoorde, Belgium) to this study (ClinicalTrials.gov Identifier: NCT01062633) is gratefully acknowledged.

REFERENCES

1. Mäkinen KK, Scheinin A. Turku sugar studies VII. Principal biochemical findings on whole saliva and plaque. *Acta Odontologica Scandinavica* 1975;**33**:129–71.
2. Mäkinen KK, Chen CY, Mäkinen PL, Bennett CA, Isokangas PJ, Isotupa KP, *et al.* Properties of whole saliva and dental plaque in relation to 40-month consumption of chewing gums containing xylitol, sorbitol or sucrose. *Caries Research* 1996;**30**:180–8.
3. Honkala S, Runnel R, Saag M, Olak J, Nõmmela R, Russak S, *et al.* Effect of erythritol and xylitol on dental caries prevention in children. *Caries Research* 2013;**7**. Submitted to Caries Research 7/2013.
4. Honkala E, Runnel R, Honkala S, Olak J, Vahlberg T, Saag M, *et al.* Measuring dental caries in the mixed dentition by ICDAS. *International Journal of Dentistry* 2011.

http://dx.doi.org/10.1155/2011/150424. Available at: http://www.hindawi.com/journals/ijd/2011/150424/.
5. Runnel R, Honkala S, Honkala E, Olak J, Nõmmela R, Vahlberg T, *et al.* Caries experience in the permanent dentition among first- and second-grade schoolchildren in southeastern Estonia. *Acta Odontologica Scandinavica* 2013;**71**:410–5.
6. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, *et al.* The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dentistry Oral Epidmiology* 2007;**35**:170–8.
7. Mäkinen KK, Saag M, Isotupa KP, Olak J, Nõmmela R, Söderling E, *et al.* Similarity of the effects of erythritol and xylitol on some risk factors of dental caries. *Caries Research* 2005;**39**:207–15.
8. Mäkinen KK, Isotupa KP, Kivilompolo T, Mäkinen PL, Murtomaa S, Petäjä J, *et al.* The effect of polyol-combinant saliva stimulants on *S. mutans* levels in plaque and saliva of patients with mental retardation. *Special Care in Dentistry* 2002;**22**:187–93.
9. Mäkinen KK. Sugar alcohols, caries incidence, and remineralization of caries lesions: a literature review. *International Journal of Dentistry* 2010. http://dx.doi.org/10.1155/2010/981072. Available at: http://www.hindawi.com/journals/ijd/2010/981072/.
10. de Cock P. Erithritol. In: O'Donnell K, Kearsley MW, editors. *Sweeteners and sugar alternatives in food technology*. 2nd ed. Wiley-Blackwell: Oxford; 2012. p. 215–41. doi:10.1002/9781118373941.ch10.
11. den Hartog GJM, Boots AW, Adam-Perrot A, Brouns F, Verkoojen IW, Weseler AR, *et al.* Erythritol is a sweet antioxidant. *Nutrition* 2010;**26**:449–58.
12. Dowd SE, Sun Y, Smith E, Kennedy JP, Jones CE, Wolcott R. Effects of biofilm treatments on the multi-species Lubbock wound biofilm model. *Journal of Wound Care* 2009;**18**(508):510–2.
13. Yao J, Zhang JL, Wu YQ, Lu ZJ. Contrasting study of erythritol and xylitol on *Streptococcus mutans*. 2009;**27**:603–5. [in Chinese, abstract in English].
14. Elseviers M, Wannemaeker B, Röper H. A sweet tooth? Dental plaque and the use of anti-cariogenic sweeteners. *Agro Food Industry Hi-Tech* 2000;**11**:24–9.
15. Kawanabe J, Hirasawa M, Takeuchi T, Oda T, Ikeda T. Noncariogenicity of erythritol as a substrate. *Caries Research* 1992;**26**:358–62.
16. Kõll-Klais P, Mändar R, Leibur E, Kjaeldgaard M. High levels of salivary lactobacilli in Estonian schoolchildren. *European Journal of Pediatric Dentistry* 2004;**5**:107–9.
17. Mäkinen KK, Bennett CA, Hujoel PP, Isokangas PJ, Isotupa KP, Pape HR, *et al.* Xylitol chewing gums and caries rates: a 40-month cohort study. *Journal of Dental Research* 1995;**74**:1904–13.
18. Mäkinen KK, Chen C-Y, Mäkinen P-L, Bennett CA, Isokangas PJ, Isotupa KP, *et al.* Properties of whole saliva and dental plaque in relation to 40-month consumption of chewing gums containing xylitol, sorbitol or sucrose. *Caries Research* 1996;**30**:180–8.
19. Mäkinen KK, Alanen P, Isokangas P, Isotupa K, Söderling E, Mäkinen PL, *et al.* Thirty-nine-month xylitol chewing-gum programme in initially 8-year old school children: a feasibility study focusing on mutans streptococci and lactobacilli. *International Journal of Dentistry* 2008;**58**:41–50.
20. Carr CJ, Krantz JC. Metabolism of the sugar alcohols. *Advances in Carbohydrate Chemistry and Biochemistry* 1945;**1**:175–92.
21. Rose RK, Dibdin GH, Shellis PR. A quantitative study of calcium binding and aggregation in selected oral bacteria. *Journal of Dental Research* 1993;**72**:78–84.